

Effects of Perinatal PBDE Exposure on Hepatic Phase I, Phase II, Phase III, and Deiodinase 1 Gene Expression Involved in Thyroid Hormone Metabolism in Male Rat Pups

David T. Szabo,^{*1} Vicki M. Richardson,[†] David G. Ross,[†] Janet J. Diliberto,[†] Prasada R.S. Kodavanti,[‡] and Linda S. Birnbaum[†]

^{*}University of North Carolina Curriculum in Toxicology, United States Environmental Protection Agency, Research Triangle Park, North Carolina 27711;

[†]Experimental; and [‡]Neurotoxicology Divisions, United States Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Research Triangle Park, North Carolina 27711

Received July 15, 2008; accepted October 7, 2008

Previous studies demonstrated that perinatal exposure to polybrominated diphenyl ethers (PBDEs), a major class of brominated flame retardants, may affect thyroid hormone (TH) concentrations by inducing hepatic uridinediphosphate-glucuronosyltransferases (UGTs). This study further examines effects of the commercial penta mixture, DE-71, on genes related to TH metabolism at different developmental time points in male rats. DE-71 is predominately composed of PBDE congeners 47, 99, 100, 153, 154 with low levels of brominated dioxin and dibenzofuran contaminants. Pregnant Long-Evans rats were orally administered 1.7 (low), 10.2 (mid), or 30.6 (high) mg/kg/day of DE-71 in corn oil from gestational day (GD) 6 to postnatal day (PND) 21. Serum and liver were collected from male pups at PND 4, 21, and 60. Total serum thyroxine (T₄) decreased to 57% (mid) and 51% (high) on PND 4, and 46% (mid) dose and 25% (high) on PND 21. Cyp1a1, Cyp2b1/2, and Cyp3a1 enzyme and mRNA expression, regulated by aryl hydrocarbon receptor, constitutive androstane receptor, and pregnane xenobiotic receptor, respectively, increased in a dose-dependent manner. UGT-T₄ enzymatic activity significantly increased, whereas age and dose-dependent effects were observed for Ugt1a6, 1a7, and 2b mRNA. Sult1b1 mRNA expression increased, whereas that of transthyretin (Ttr) decreased as did both the deiodinase I (D1) enzyme activity and mRNA expression. Hepatic efflux transporters Mdr1 (multi-drug resistance), Mrp2 (multidrug resistance-associated protein), and Mrp3 and influx transporter Oatp1a4 mRNA expression increased. In this study the most sensitive responses to PBDEs following DE-71 exposure were CYP2B and D1 activities and Cyb2b1/2, d1, Mdr1, Mrp2, and Mrp3 gene expression. All responses were reversible by PND 60. In conclusion, deiodination, active transport, and sulfation, in addition to glucuronidation, may be involved in disruption of TH homeostasis due to perinatal exposure to DE-71 in male rat offspring.

Key Words: PBDE; flame retardant; thyroid; hepatic metabolism; rat.

Brominated flame retardants (BFRs) are added to consumer products to delay the ignition and burning of materials, thereby saving lives and reducing property damage. Polybrominated diphenyl ethers (PBDEs), a major type of BFRs, consist of a family of 209 possible congeners; however, what is actually used commercially is a flame-retardant mixture. Three PBDE commercial flame-retardant products have been produced which are mixtures of congeners, and referred to based on the average level of bromination in the mixture: decabrominated diphenyl ether (e.g., DE-83R); octabrominated diphenyl ether, for example, (DE-79); and pentabrominated diphenyl ether (e.g., DE-71). DE-71 is used in polyurethane foams, upholstery, car and airline seats, office and household furniture, carpet pads, mattresses, and pillows (Birnbaum *et al.*, 2004). DE-71 consists primarily of the congeners BDE 47, 99, 100, 153, 154 (LaA Guardia *et al.*, 2006) and has been found to contain varying low levels of polybrominated dibenzop-dioxins and dibenzofurans (Hanari *et al.*, 2006). This commercial penta mixture was used as an additive flame retardant which allows it to escape into the environment (Kim *et al.*, 2006) including household dust (Stapleton *et al.*, 2005). The European Union has banned production and use of all PBDE mixtures. Although, the sole U.S. producer voluntarily stopped production of DE-71 (penta) and DE-79 (octa) at the end of 2004 and 11 states have banned their production, use and import, the presence of PBDEs in the environment and biota continues. The persistence of PBDEs is attributed to its lipophilic properties and bioaccumulative nature.

PBDEs have been detected in human serum, breast milk, and adipose tissue at varying levels (Birnbaum *et al.*, 2004; McDonald, 2002). PBDEs, as polychlorinated biphenyls (PCBs) and dioxins, are structurally similar to thyroid hormones (THs) and therefore, may act as endocrine disruptors via alterations in TH homeostasis (Darnerud *et al.*, 2001; Hooper and McDonald, 2000; Kuriyama *et al.*, 2007; Tseng *et al.*, 2008). As reviewed by Costa and Giordano (2007), PBDEs have also been identified as developmental neuro-toxicants. A single low exposure of BDE 47, 99, and 209 to

¹ To whom correspondence should be addressed at United States Environmental Protection Agency, 109 TW Alexander Dr., MD B143-01, Research Triangle Park, NC 27711. Fax: (919) 541-9464. E-mail: Szabo.David@epa.gov.

mice during neonatal development permanently alters behavior (Eriksson *et al.*, 2001; Rice *et al.*, 2007; Viberg *et al.*, 2003).

TH has a crucial role in fetal brain development in both animals and humans. During fetal and early neonatal periods, disorders of TH homeostasis may result in motor and cognitive disorders (Sher *et al.*, 1998). The thyroid axis contains numerous sites in which xenobiotics can alter the hormonal balance. Decreases in TH concentrations are often observed in rodents with increases in hepatic thyroxine (T_4) glucuronidation leading to increased biliary elimination of the conjugated hormone (Barter and Klaassen, 1992; Vansell and Klaassen, 2002). Maternal administration of DE-71 during gestation and lactation results in a reduction and functional disruption of pup serum T_4 levels with increase in uridinediphosphate-glucuronosyltransferases (UGTs) activity in rats (Ellis-Hutchings *et al.*, 2006; Zhou *et al.*, 2001). Adult rats exposed to PBDEs also have reduced concentration of circulating T_4 which is associated with an induction in hepatic UGTs (Hallgren and Darnerud, 2002). A 14-day exposure to BDE 47 at 18 mg/kg/day reduced total serum T_4 concentrations in rats (Darnerud and Sinjari, 1996). Additionally, a 4-day exposure in female mice resulted in 50% decrease in circulating total T_4 concentrations at 100 mg/kg/day of BDE 47 (Richardson *et al.*, 2008). Collectively, these studies demonstrate that DE-71 and individual PBDE congeners alter TH homeostasis in developing and adult rodents.

Although it has been suggested that decreases in circulating THs may be due to induction of UGT, it is unclear if this alone is responsible for the decrease in circulating TH. UGT1A-deficient Gunn rats exposed to phenobarbital (PB) or PCBs had decreases in serum total T_4 demonstrating a lack of total dependence on glucuronidation (Collins and Capen, 1980; Kato *et al.*, 2004). Sulfotransferases (SULTs) also play a key role in TH homeostasis, as they are the major pathway for T_4 conjugation in humans (Visser, 1994). THs regulate SULT gene expression in an isoform-specific manner (Dunn and Klaassen, 2000). Studies using adult rat hepatocytes demonstrated SULT1B1 and 1C1 conjugate iodothyronines (Kester *et al.*, 2003). Furthermore, sulfation increases the degradation of T_4 by type I iodothyronine deiodinase (D1) (Kaptein *et al.*, 1997). D1 is a microsomal selenoenzyme which catalyzes deiodination of the prohormone T_4 to the active 3,5,3'-triiodothyronine (T_3) as well as to the inactive metabolite reverse- T_3 (rT_3), and subsequently to the inactive metabolite 3,3'-diiodothyronine (T_2) (Visser, 1994). These studies suggest sulfation and deiodination, along with glucuronidation, may play a role in altering the homeostasis of TH (Hood and Klaassen, 2000). Further, *in vivo* studies show that PCB metabolites (Kato *et al.*, 2004) and hydroxylated PBDEs (Meerts *et al.*, 2000) can bind to transthyretin (Ttr), a major TH transport protein in plasma, which also may cause a decrease in serum total T_4 .

Phase I, II, and III xenobiotic metabolizing enzymes (XMEs) play fundamental roles in metabolism and elimination of xenobiotics. XMEs can be present either at the basal level and/

or are induced or inhibited after exposure to xenobiotics via activation of a variety of nuclear receptors including aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), and pregnane xenobiotic receptor (PXR) (Tompkins and Wallace, 2007; Xu *et al.*, 2005). Recent studies show that BDE 47 predominantly activates hepatic CAR and to a lesser extent PXR regulated genes following a 4-day exposure in adult mice (Pacyniak *et al.*, 2007; Richardson *et al.*, 2008). The role of UGTs in the DE-71 and individual PBDE-mediated decrease in TH has been examined in the adult and developing rodent, however, the role of other phase II metabolizing enzymes (i.e., SULTs) and phase III transporters in the reduction of TH has yet to be examined. A correlation between induction of hepatic UGT mRNAs, multidrug resistance-associated protein (Mrp2) mRNA, and organic anion transporting protein (Oatp1a4) mRNA levels, with decreases in serum TH concentrations has been observed (Hagenbuch, 2007; Mitchell *et al.*, 2005; Ribeiro *et al.*, 1996). P-glycoprotein (Pgp), a product of the multidrug resistance (Mdr) gene, is expressed in various tissues and plays an important role in drug absorption and disposition, whereas alterations in the expression levels of Pgp are involved in the variability of pharmacokinetics of many drugs and xenobiotics. There is evidence that multidrug-resistance transporters are important in unconjugated TH efflux (Mitchell *et al.*, 2005). In a recent study, BDE-47 altered Mdr1a and Mrp3 mRNA expression levels in adult mice (Richardson *et al.*, 2008). Studies using *Xenopus laevis* oocytes identified transporters involved in TH uptake; Na(+) taurocholate cotransporting polypeptide (NTCP), and the organic anion transporting polypeptide (OATP) (Friesema *et al.*, 2005). Collectively, these studies indicate that active transport, in addition to sulfation, deiodination, and altered serum binding, could also play a role in the disposition of TH.

This study examines how perinatal exposures to DE-71 alter the expression of hepatic genes involved in TH homeostasis in Long-Evans male rat pups in order to further the risk assessment of this commercial penta-BDE mixture. In addition to cytochrome P450s (CYPs) and UGTs mRNA expression and enzyme activity, SULTs, D1, and transporters were also examined to determine other possible mechanisms involved in disruption of TH homeostasis following early life exposure to DE-71. The basal expression of major XMEs and transporters in the developing rat pups is also described (Supplementary Data).

METHODS AND MATERIALS

Animals. Time-pregnant Long-Evans rats, approximately 80–90 days of age, were obtained from Charles River Laboratories, Inc. (Raleigh, NC) on GD 2, and allowed 4 days acclimation in an American Association for Accreditation of Laboratory Animal Care–approved animal facility prior to being treated. Dams were housed individually in plastic cages (45 × 24 × 20 cm) with sterilized pine shavings as bedding, which was changed twice a week except on the day of parturition (i.e., GD 21). Rats were provided with Purina 5001 Rodent Chow (Ralston Purina Co., St Louis, MO) and tap water

ad libitum. On PND 21, offspring were counted, sexed, and group-weighted by sex. Average pup weight by sex was calculated by dividing the group weight by the number of pups. Pups were weaned on PND 21, and housed by gender in groups of two per cage.

Chemicals and treatment. DE-71 (penta-BDE, lot 75500K20A) was generously supplied by the Great Lakes Chemical Corporation (West Lafayette, IN). DE-71 is a mixture that consists primarily of tetra and penta congeners. The stock DE-71 solution (300 mg/ml) was prepared by mixing the compound with corn oil and sonicating for 30 min at 40°C. The desired dosing solutions were obtained by serial dilution with corn oil. The dams were orally dosed (2 ml/kg), via gavage, with DE-71 (0, 1.7, 10.2, or 30.6 mg/kg/day) from GD 6 through PND 21, except for PND 0 (day of birth) when dams were left undisturbed. On PND 4, litters were culled to eight pups per litter, four males and four females. Pups were euthanized on PND 4, 21, or 60 with CO₂ asphyxiation followed by exsanguinations via cardiac puncture where blood and liver were collected. Liver samples were removed immediately and frozen in liquid nitrogen. Serum was obtained after clotting whole blood on ice, followed by centrifugation at 12000 g at 4°C for 20 min. All serum and liver samples for each age point were obtained from a minimum of eight litters, and were stored at -80°C until analysis for TH (T₄, T₃) concentrations, hepatic enzyme activity, and mRNA expression analysis. The liver was the experimental unit.

TH assay. Serum concentrations of total T₄ and T₃ were measured in duplicate as previously described (Goldey *et al.*, 1995) by using standard radioimmunoassay kits (Diagnostic Products Corp., Los Angeles, CA).

Hepatic enzyme activity assay. Liver microsomal fractions were prepared as described previously (DeVito *et al.*, 1993). Microsomal protein concentrations were determined using the Bio-Rad protein assay kit (Bio-Rad, Richmond, CA) with bovine serum albumin (BSA) (Sigma, St Louis, MO) as the standard. Hepatic microsomal ethoxyresorufin-*O*-dealkylase (EROD) activity (a marker for CYP1A1 activity), pentoxoresorufin-*O*-dealkylase (PROD) activity (a marker of CYP2B activity), and benzyloxyresorufin-*O*-dealkylase (BROD) activity (a marker of CYP3A activity) were assayed according to DeVito *et al.* (1993). All substrate concentrations were 1.5 nM. EROD, PROD, and BROD values were calculated as pmol resorufin per mg protein per min.

UGT-T₄ activity assay. UGT-T₄ activity was determined by using a modified assay (Zhou *et al.*, 2002) based on a previously published method (Visser *et al.*, 1993). A 100-μl aliquot of microsomes (2 mg protein per 100 mM Tris/HCl buffer pH 7.4) was incubated at 37°C with purified ¹²⁵I-T₄ (NEN Life Science Products, Inc., Boston, MA), 6-n-propyl-2-thiouracil (PTU), and uridine diphosphoglucuronic acid (UDPGA) (or no UDPGA for blank) (Sigma) over a 30-min period. The reaction was stopped with methanol (Sigma) followed by centrifugation and mixing the supernatant with 0.1 M HCl (Sigma). The formed glucuronide T₄ was separated by chromatography on 2 ml of lipophilic sephadex (Sigma) LH-20 columns (Superlco, Bellefonte, PA) and radioactivity determined by gamma scintillation spectrometry.

Outer-ring deiodinase activity. D1 was determined by quantifying the amount of I¹²⁵ released from I¹²⁵-rT₃ (NEN) (Leonard and Rosenberg, 1980). All reactions contained 75 μl of 0.1 M phosphate (pH 7.0) and 2 mM ethylenediaminetetraacetic acid (EDTA) buffer (Sigma), 25 μl of 2 mg/ml microsomal protein, and 1 mM PTU (Sigma) (in blanks to determine spontaneous deiodination). The reaction was stopped by adding 50 μl of 4% BSA (Sigma) in 1 mM PTU solution. Ten percent trichloroacetic acid (TCA; Sigma) was added, centrifuged, and the supernatant was loaded on 2 ml of lipophilic sephadex (Sigma) in a LH-20 column (Superlco) (Rooda *et al.*, 1987). Free iodine was eluted using 0.1 M HCl (Sigma) and radioactivity determined as above.

SULT activity. Iodothyronine SULT activities were assayed by incubation of 1 μM of T₄ and 10⁵ cpm of ¹²⁵I-labeled T₄ (NEN) for 30 min at 37°C with 20–40 μg protein/ml of liver cytosol in the presence or absence (blank) of

50 μM 3'-phosphoadenosine 5'-phosphosulfate (PAPS; Sigma) in 0.2 ml of 0.1 M phosphate (pH 7.2) and 2 mM EDTA (Sigma) (Kaptein *et al.*, 1997). The mixtures were applied to 1 ml of lipophilic sephadex (Sigma) LH-20 minicolumns (Superlco), and equilibrated in 0.1 M HCl (Sigma). Iodine, sulfated iodothyronines, and nonsulfated iodothyronines were successively eluted with 2 × 1 ml of 0.1 M HCl, 6 × 1 ml of ethanol/water (20/80, vol/vol), and 3 × 1 ml of ethanol/0.1 M NaOH (50:50, vol/vol) (Sigma), respectively. Fractions were collected and measured for radioactivity.

RNA isolation and real-time RT-PCR analysis. Total RNA was isolated using the RNeasy Midi Kit with DNase I digestion performed during column purification (Qiagen, Hilden, Germany). To assess the integrity of the RNA samples, the 2100 Bioanalyzer was used (Agilent Technologies, Palo Alto, CA) with RNA Integrity Numbers (RINs) greater than 8.1. Samples with RINs less than 8.1 were not analyzed by RT-PCR (*n* = 5 per group). Real-time RT-PCR was performed using the ABI Prism 7700 Sequence Detection System (ABI, Foster City, CA). One-hundred nanograms of total RNA was used for each reaction. cDNA was synthesized using TaqMan Reverse Transcriptase Kits (ABI, Foster City, CA). PCR was then performed on all cDNAs using TaqMan Universal PCR Master Mix and TaqMan Gene Expression Assays (ABI) unless otherwise indicated. The probe/primer sets are Cyp1a1 (Rn00487218_m1), Cyp3a1 (Rn01412959_g1), Ugt1a1 (Rn007549447_m1), Ugt1a6 (Rn00756113_m1), Ugt1a7 (Rn01643133_g1), Ugt2b (Rn0234965_m1), Sult1a1 (Rn01510633_m1), Sult1b1 (Rn01524591_g1), Sult1c1 (Rn01528019_m1) Mdr1 (Rn01529259_m1), Mrp2 (Rn00680936_m1), Mrp3 (Rn00589786_m1), Oatp1a4 (Rn00584891_m1), Oat2 (Rn00585513_m1), Ntcp (Rn00690543_m1), Dio1 (Rn00562124_m1), Ttr (Rn01416940_m1), Cyp2b1 (Fw-5'GTGGGCCA-AGCTGAGGAT3', Rev-5'GAGAATCGCCGAAGGGC3', Probe-5'ATCG-CTGTGATTGAGCCAATCTTCAAGG3') and Cyp2b2 (Fw-TTCTGCG-CATGGAGAAAGTG3', Rev-5'GATCATGAGGTTCTCATGATGGAA3', Probe-5'CCTGCATGGATGAGAGAGAGAGAGTCG3'). The thermal cycle condition for the RT reaction was as follows: 10 min at 25°C, 30 min at 48°C, and then 5 min at 95°C. The PCR reaction was performed as follows: 2 min at 50°C (uracil-DNA glycosylase activation), 10 min at 95°C (activation of *Taq* DNA polymerase), and 40 cycles of denaturation at 95°C for 15 s followed by annealing and extension at 60°C for 1 min. Data were analyzed using the Sequence Detection Systems software (ABI). All RT-PCR data were quantified by the ΔΔC_t method (Applied Biosystems User Bulletin 2). These data were compared with a calibrator sample (a test sample where all other samples are relative expression levels of it) and an endogenous control (18S). In the ΔΔC_t method, the control group is used as a reference point for all other dose groups; therefore, all samples are expressed as fold difference as compared with control.

Data analysis. The statistical intergroup comparisons of T₄, T₃, RT-PCR, and enzyme activity data were determined by using a two-way ANOVA (examining the effect of dose, age, and their interaction on response) with a follow up contrast of dose to control in each age group. The follow up contrast were Bonferroni adjusted. The levels of probability of statistical significance are *p* < 0.05 and 0.01.

RESULTS

Serum T₄ and T₃ Levels

A significant interaction between age and dose (*F*_{6,48} = 21.56; *p* < 0.001) was observed for total serum T₄ (Table 1). A *post hoc* test indicated no significant effects at the low dose for any time points tested. However, at PND 4, exposure to DE-71 decreased total serum T₄ to 57 and 51% of control levels with exposure to 10.2 and 30.6 mg/kg/day, respectively. There was also a decrease of total serum T₄ at PND 21 with levels 46 and 25% of controls with perinatal exposure to 10.2 and 30.6 mg/kg/day, respectively. By PND 60, no differences

TABLE 1
Circulating Total T₄ and T₃ Levels (ng/ml) in Male Pups during Perinatal Exposure to a Penta PBDE Mixture, DE-71

DE-71	PND		
	4	21	60
Total T ₄ levels			
0 mg/kg/day	17.15 ± 0.58	53.18 ± 2.57	71.14 ± 4.68
1.7 mg/kg/day	18.73 ± 1.74	47.68 ± 3.11	67.96 ± 7.73
10.2 mg/kg/day	9.80 ± 0.52*	24.68 ± 1.26*	72.05 ± 11.21
30.6 mg/kg/day	8.46 ± 0.55*	13.64 ± 0.93*	68.13 ± 5.98
Total T ₃ levels			
0 mg/kg/day	0.26 ± 0.01	1.08 ± 0.06	0.90 ± 0.05
1.7 mg/kg/day	0.28 ± 0.02	1.14 ± 0.05	0.76 ± 0.05
10.2 mg/kg/day	0.27 ± 0.01	0.90 ± 0.04	0.77 ± 0.05
30.6 mg/kg/day	0.29 ± 0.01	0.88 ± 0.04	0.83 ± 0.05

Note. T₄ and T₃ serum levels were analyzed from male rats at PND 4, 21, and 60 after perinatal exposure to the polybrominated diphenyl ether mixture, DE-71 (0, 1.7, 10.2, 30.6 mg/kg/day) from GD 6 to PND 21. Significantly different from control: **p* < 0.05. Data are expressed as mean ± SD, *n* = 7–9 rats.

between control and dose groups were seen. Interestingly, there was no significant effect of dose on serum total T₃ levels at any age group. In this study, decreases in total T₄ levels were similar in both males and females. Therefore, we selected males only for understanding the mechanism as female offspring from this cohort were used for reproductive and neurotoxicological endpoints (Kodavanti *et al.*, unpublished data).

Cytochrome P450s: Enzyme Activity and Gene Expression

Cyp1a1 mRNA expression and EROD activity are used as markers for Ah receptor activation (Table 2). There was a significant interaction of age and dose ($F_{6,48} = 37.16$; *p* < 0.0001) for Cyp1a1. A *post hoc* test indicates an effect at all doses tested at PND 4 and PND 21, and only for 30.6 mg/kg/day at PND 60. Cyp1a1 mRNA expression was induced at the 1.7, 10.2, and 30.6 mg/kg/day treatment doses by 16.1-, 44.3-, 154.2-fold at PND 4, and 4.6-, 10.2-, and 20.1-fold at PND 21, respectively. There remained a residual 5.6-fold increase in Cyp1a1 mRNA expression with the 30.6 mg/kg/day treatment group at PND 60. In agreement with this, EROD indicated a significant interaction of age and dose ($F_{6,48} = 152.3$; *p* < 0.0001) at all doses tested for PND 4 and PND 21, but not at PND 60. Hepatic EROD increased at the 1.7, 10.2, and 30.6 mg/kg/day doses by 23.3-, 49.5-, and 215.3-fold at PND 4, and 4.1-, 17.1-, and 31.9-fold at PND 21, respectively.

Cyp2b1 and Cyp2b2 mRNA expression and PROD activity are used as markers for CAR receptor activation (Maglich *et al.*, 2002; Waxman, 1999; Yamada *et al.*, 2006). There was a significant interaction of age and dose for Cyp2b1 ($F_{6,48} = 33.23$; *p* < 0.0001) and for Cyp2b2 ($F_{6,48} = 41.28$; *p* < 0.0001). A *post hoc* test indicated an effect at all doses tested at PND 4 and 21 but not at PND 60. Hepatic Cyp2b1 mRNA

TABLE 2
Effect of DE-71 on Hepatic Cytochrome P450 Gene Expression and Protein Activity

Target	Treatment (mg/kg/day)	PND 4	PND 21	PND 60
Cyp1a1 ^a	0	1.6 ± 0.2	8.2 ± 2.5	0.02 ± 0.0
	1.7	26.0 ± 9.9*	38.1 ± 11.6**	0.02 ± 0.0
	10.2	71.3 ± 19.7*	84.1 ± 24.8*	0.04 ± 0.0
	30.6	248.3 ± 29*	165.8 ± 55.6*	0.10 ± 0.1**
EROD ^b	0	4.2 ± 1.1	108.9 ± 12	87.2 ± 9.0
	1.7	98.2 ± 13.4**	443.2 ± 50.4**	84.3 ± 7.2
	10.2	208.1 ± 62.3**	1872.0 ± 203.2**	83.2 ± 5.1
	30.6	904.1 ± 113.2**	3472.2 ± 439.1**	85.7 ± 6.8
Cyp2b1 ^a	0	1.7 ± 0.1	5.5 ± 0.8	0.3 ± 0.0
	1.7	6.6 ± 2.7**	37.7 ± 17.3**	0.4 ± 0.2
	10.2	21.6 ± 7.4**	89.7 ± 33.2*	0.5 ± 0.1
	30.6	33.7 ± 6.8*	147.7 ± 20.1*	0.5 ± 0.1
Cyp2b2 ^a	0	1.7 ± 0.3	6.5 ± 1.5	0.7 ± 0.1
	1.7	3.4 ± 1.74	27.5 ± 5.6**	1.0 ± 0.3
	10.2	9.6 ± 3.5**	42.8 ± 9.8**	1.0 ± 0.2
	30.6	12.1 ± 2.7*	69.6 ± 13.2*	0.9 ± 0.1
PROD ^b	0	0.4 ± 0.2	21 ± 7.2	11.2 ± 4.2
	1.7	3.5 ± 1.2**	108 ± 12.4**	12.3 ± 3.1
	10.2	18.9 ± 5.5*	219 ± 48.2*	13.2 ± 2.9
	30.6	22.2 ± 6.7*	202 ± 42.8*	11.7 ± 4.6
Cyp3a1 ^a	0	1.5 ± 1.1	1.1 ± 0.8	0.5 ± 0.1
	1.7	1.0 ± 0.4	1.2 ± 0.8	0.6 ± 0.1
	10.2	2.4 ± 1.0	7.6 ± 2.6**	0.7 ± 0.1
	30.6	3.7 ± 2.2**	16.3 ± 5.8*	0.6 ± 0.1
BROD ^b	0	15.1 ± 11.2	52.1 ± 22.8	25.3 ± 10.1
	1.7	35.0 ± 10.7	301.0 ± 100.7**	25.9 ± 11.2
	10.2	148.3 ± 42.0**	1818.4 ± 420.1*	26.2 ± 12.8
	30.6	201.1 ± 82.5*	2120.5 ± 582.5*	26.3 ± 11.1

Note. Phase I hepatic microsomal EROD, PROD, and BROD activity along with real-time PCR of Cyp1a1, Cyp2b1, Cyp2b2, and Cyp3a1 gene expression were measured. Microsomes and RNA were isolated from male rat livers at PND 4, 21, and 60 after perinatal exposure to the polybrominated diphenyl ether mixture, DE-71 (0, 1.7, 10.2, and 30.6 mg/kg/day) from GD 6 to PND 21. Significantly different from control: (*p* < 0.01)* or (*p* < 0.05)**.

^aData are expressed as relative quantitation mean ± SD.

^bData are expressed as pmol/mg protein/min ± SD, *n* = 5.

expression increased at the 1.7, 10.2, and 30.6 mg/kg/day doses by 4-, 13-, and 20.2-fold at PND 4, and 6.8-, 16.3-, and 26.8-fold at PND 21, respectively. Hepatic Cyp2b2 mRNA expression also increased significantly at the 1.7, 10.2, and 30.6 mg/kg/day doses by 1.9-, 5.6-, and 7-fold at PND 4, and 2.7-, 6.6-, and 10.6-fold at PND 21, respectively. In agreement, PROD showed a significant interaction of age and dose ($F_{6,48} = 33.53$; *p* < 0.0001) with at all doses tested at PND 4 and PND 21, but not PND 60. Hepatic PROD, a marker for overall CYP2B activity, increased at the 1.7, 10.2, and 30.6 mg/kg/day doses by 8.8-, 47.2-, and 55.5-fold at PND 4, and 5.1-, 10.4-, and 9.6-fold at PND 21, respectively.

Cyp3a1 mRNA expression and BROD activity were used as markers for PXR receptor activation (Xie *et al.*, 2000). There was a significant interaction of age and dose for Cyp3a1

TABLE 3
Effect of DE-71 on Hepatic UGT-T₄ Protein Activity and Gene Expression

Target	Treatment (mg/kg/day)	PND 4	PND 21	PND 60
UGT-T ₄ ^a	0	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
	1.7	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.0
	10.2	0.7 ± 0.1	0.7 ± 0.2	0.5 ± 0.1
	30.6	1.5 ± 0.3**	1.7 ± 0.4**	0.5 ± 0.1
Ugt1a1 ^b	0	1.2 ± 0.4	0.2 ± 0.1	0.1 ± 0.1
	1.7	1.7 ± 0.5	0.2 ± 0.1	0.1 ± 0.0
	10.2	1.7 ± 0.4	0.2 ± 0.0	0.1 ± 0.0
	30.6	1.5 ± 0.2	0.1 ± 0.0	0.1 ± 0.0
Ugt1a6 ^b	0	1.8 ± 1.0	229.4 ± 12.1	79.7 ± 16.0
	1.7	3.4 ± 1.3	245.2 ± 34.2	98.3 ± 16.2
	10.2	12.8 ± 4.8**	229.5 ± 17.8	108.7 ± 16.9
	30.6	34.0 ± 10.5*	201.1 ± 30.2	112.1 ± 17.8
Ugt1a7 ^b	0	1.8 ± 0.2	0.3 ± 0.1	0.003 ± 0.0003
	1.7	2.5 ± 0.5	0.3 ± 0.1	0.003 ± 0.0005
	10.2	2.9 ± 0.8**	0.4 ± 0.1	0.003 ± 0.0002
	30.6	3.8 ± 1.5**	0.7 ± 0.2*	0.004 ± 0.0005
Ugt2b ^b	0	1.2 ± 0.4	1.2 ± 0.7	7534 ± 2963
	1.7	1.0 ± 0.4	7.6 ± 6.0	10,043 ± 3581
	10.2	1.3 ± 1.0	1.5 ± 0.3	9881 ± 2274
	30.6	6.5 ± 2.2*	78.0 ± 9.3*	11,318 ± 2227

Note. Phase II hepatic microsomal enzyme activity was measured for UGTs using T₄ as a substrate along with real-time PCR of Ugt1a1, Ugt1a6, Ugt1a7, and Ugt2b gene expression. Microsomes and RNA were isolated from male rat livers at PND 4, 21, and 60 after perinatal exposure to the polybrominated diphenyl ether mixture, DE-71 (0, 1.7, 10.2, and 30.6 mg/kg/day) from GD 6 to PND 21. Significantly different from control: ($p < 0.01$)* or ($p < 0.05$)**.

^aData are expressed as pmol/mg protein/min ± SD, $n = 5$.

^bData are expressed as relative quantitation mean ± SD.

($F_{6,48} = 17.66$, $p < 0.0001$). A *post hoc* test indicated a significant effect at all doses tested at PND 4 and PND 21, but not at PND 60. Hepatic Cyp3a1 mRNA expression increased with 30.6 mg/kg/day treatment by 2.6-fold at PND 4 and 6.9 and 14.7-fold at PND 21 with 10.2 and 30.6 mg/kg/day treatment, respectively. In agreement with this, a significant interaction of age and dose ($F_{6,48} = 64.90$; $p < 0.0001$) was observed for BROD at all doses tested at PND 4 and 21. BROD increased 9.8- and 13.3-fold at PND 4 with treatments of 10.2 and 30.6 mg/kg/day, and 5.8-, 34.9-, and 40.7-fold at PND 21 with treatments of 1.7, 10.2, and 30.6 mg/kg/day, respectively.

UGTs: Enzyme Activity and Gene Expression

A significant interaction of age and dose ($F_{6,48} = 12.81$; $p < 0.0001$) was detected for UGT-T₄ enzyme activity (Table 3). A *post hoc* test indicated a significant effect of DE-71 at the highest dose with a 2.5- and 2.8-fold increase at PND 4 and 21, respectively. No induction at PND 60 was observed. To determine which UGT isoform(s) may contribute to the overall enzyme activity, hepatic UGT mRNA expressions were further examined.

For Ugt1a1 mRNA expression, a significant interaction of age ($F_{6,48} = 4.755$; $p < 0.0001$) but not dose was observed at every dose group. However, a significant interaction of age and dose for Ugt1a6 mRNA expression ($F_{6,48} = 4.755$; $p < 0.0001$) was identified at 10.6 and 30.6 mg/kg/day, with an increase of 7.2- and 19.6-fold at PND 4, respectively. Additionally, there was a significant interaction of age and dose for Ugt1a7 mRNA expression ($F_{6,48} = 3.479$; $p < 0.0062$) with an increase of 1.6- and 2.1-fold at 10.6 and 30 mg/kg/day, respectively, on PND 4 and 2.6-fold at the high dose on PND 21. Lastly, a significant effect of age for Ugt2b mRNA expression ($F_{6,48} = 10.34$; $p < 0.0001$) was identified. The large increase in developmental expression observed for Ugt2b may possibly mask a dose effect; therefore a one-way ANOVA was performed which identified a significant increase with 30.6 mg/kg/day treatment of 5.5- and 67.6-fold at PND 4 and 21, respectively.

Thus, although a significant induction of gene expression was observed at the middle dose, there is no concomitant increase in UGT-T₄ activity suggesting the enzyme assay used may not be sensitive enough to reflect the observed changes in gene expression.

SULTs: Enzyme Activity and Gene Expression

Exposure to DE-71 led to a significant effect on age for SULT-T₄ ($F_{6,48} = 11.25$; $p < 0.0001$), Sult1a1 ($F_{6,48} = 154.4$; $p < 0.0001$) and Sult1c1 ($F_{6,48} = 31.39$; $p < 0.0001$) with no dose related changes (Table 4). However, a significant interaction of age and dose was identified for Sult1b1 mRNA expression ($F_{6,48} = 7.540$; $p < 0.0001$). A *post hoc* test identified a 2.8-fold increase on PND 4 with exposure to the high dose, and 2.1- and 4.1-fold increase at 10.2 and 30.6 mg/kg/day on PND 21, respectively.

Efflux Transporters: Gene Expression

A significant interaction between age and dose for the efflux transporter Mdr1 ($F_{6,48} = 10.34$; $p < 0.0001$) was observed (Table 5). A *post hoc* test indicated an effect of DE-71 at all doses for PND 4 and 21, but not at PND 60. There was a dose-dependent increase in hepatic Mdr1 mRNA expression of 1.5-, 1.8-, and 2.4-fold at PND 4 and 1.5-, 1.8-, and 2.2-fold at PND 21. Similarly, a significant interaction of age and dose for Mrp2 mRNA expression ($F_{6,48} = 27.65$; $p < 0.0001$) was also observed. Mrp2 mRNA gene expression levels increased 1.6-fold at the 30.6 mg/kg/day treatment group for PND 4. The expression of Mrp2 at PND 21 increased 1.5-, 1.9-, and 3.4-fold, respectively. At PND 60, a significant 30 and 40% reduction at 10.2 and 30.6 mg/kg/day was also found. Lastly, a significant interaction of age and dose was observed for Mrp3 mRNA expression ($F_{6,48} = 17.62$; $p < 0.0001$). A *post hoc* test indicated an effect for all doses at PND 4 and 21, but not at PND 60. The expression of hepatic Mrp3 mRNA, a major sinusoidal efflux transporter of glucuronides, indicated a 1.6-, 2.5-, and 4.1-fold increase at PND 4 and a 2.2-, 2.8-, and 5.1-fold increase at PND 21.

TABLE 4

Effect of DE-71 on Hepatic SULT-T4 Protein Activity and Gene Expression

Target	Treatment (mg/kg/day)	PND 4	PND 21	PND 60
SULT-T ₄ ^a	0	0.2 ± 0.2	0.6 ± 0.1	0.5 ± 0.2
	1.7	0.1 ± 0.0	0.4 ± 0.3	0.5 ± 0.4
	10.2	0.1 ± 0.1	0.4 ± 0.2	0.6 ± 0.4
	30.6	0.3 ± 0.2	0.5 ± 0.3	0.6 ± 0.4
Sult1a1 ^b	0	1.0 ± 0.3	2.2 ± 0.2	0.34 ± 0.1
	1.7	1.2 ± 0.3	2.7 ± 0.5	0.35 ± 0.1
	10.2	1.1 ± 0.3	2.1 ± 0.5	0.40 ± 0.1
	30.6	1.1 ± 0.6	2.2 ± 0.6	0.39 ± 0.2
Sult1b1 ^b	0	1.1 ± 0.1	3.0 ± 0.6	2.4 ± 0.6
	1.7	1.8 ± 0.1	3.6 ± 0.2	2.1 ± 0.8
	10.2	1.1 ± 0.2	6.4 ± 0.8**	2.0 ± 0.5
	30.6	2.9 ± 0.6**	12.4 ± 1.0**	2.0 ± 0.1
Sult1c1 ^b	0	1.7 ± 0.6	4.1 ± 1.5	1.0 ± 0.1
	1.7	2.1 ± 0.5	5.0 ± 2.5	1.0 ± 0.2
	10.2	2.3 ± 0.9	3.8 ± 0.8	1.1 ± 0.5
	30.6	2.2 ± 0.4	4.5 ± 3.3	0.9 ± 0.4

Note. Phase II hepatic sulfotransferase enzyme activity was measured using T₄ as a substrate along with real-time PCR of Sult1a1, Sult1b1, and Sult1c1 gene expression. Cytosolic fractions and RNA were isolated from male rat livers at PND 4, 21, and 60 after perinatal exposure to the polybrominated diphenyl ether mixture, DE-71 (0, 1.7, 10.2, and 30.6 mg/kg/day) from GD 6 to PND 21. Significantly different from control: ($p < 0.01$)* or ($p < 0.05$)**.

^aData are expressed as pmol/mg protein/min ± SD, $n = 5$.

^bData are expressed as relative quantitation mean ± SD.

Influx Transporters: Gene Expression

A significant effect of age was observed for Oat2 ($F_{6,48} = 270.5$; $p < 0.0001$) and Ntcp ($F_{6,48} = 42.79$; $p < 0.0001$) but no dose effect was present (Table 6). However, a significant

TABLE 5

Effect of DE-71 on Hepatic Efflux Transporter Gene Expression

Target	Treatment (mg/kg/day)	PND 4	PND 21	PND 60
Mdr1 ^a	0	1.1 ± 0.1	2.3 ± 0.1	2.4 ± 0.2
	1.7	1.7 ± 0.1**	3.3 ± 0.4**	2.3 ± 0.3
	10.2	1.9 ± 0.8**	4.1 ± 0.4**	2.7 ± 0.0
	30.6	2.6 ± 0.1**	5.0 ± 0.9**	2.3 ± 0.9
Mrp2 ^a	0	0.8 ± 0.2	1.1 ± 0.1	1.0 ± 0.1
	1.7	0.9 ± 0.2	1.6 ± 0.2**	1.0 ± 0.1
	10.2	0.9 ± 0.1	2.0 ± 0.2**	0.7 ± 0.0**
	30.6	1.3 ± 0.0**	2.8 ± 0.9**	0.6 ± 0.0**
Mrp3 ^a	0	1.3 ± 0.2	2.4 ± 0.3	4.24 ± 0.4
	1.7	2.1 ± 0.0**	5.2 ± 1.1**	4.11 ± 0.6
	10.2	3.3 ± 1.7**	6.6 ± 1.6**	3.83 ± 0.3
	30.6	5.1 ± 1.1**	12.2 ± 2.3*	4.14 ± 0.9

Note. Phase III hepatic efflux transporters were measured for Mdr1, Mrp2, and Mrp3 by real-time PCR. RNA was isolated from male rat livers at PND 4, 21, and 60 after perinatal exposure to the polybrominated diphenyl ether mixture, DE-71 (0, 1.7, 10.2, and 30.6 mg/kg/day) from GD 6 to PND 21. Significantly different from control: ($p < 0.01$)* or ($p < 0.05$)**.

^aData are expressed as relative quantitation mean ± SD, $n = 5$.

TABLE 6

Effect of DE-71 on Hepatic Influx Transporter Gene Expression

Target	Treatment (mg/kg/day)	PND 4	PND 21	PND 60
Oat2 ^a	0	1.3 ± 0.3	9.5 ± 1.3	9.5 ± 0.5
	1.7	1.2 ± 0.1	8.4 ± 1.7	8.5 ± 1.9
	10.2	1.5 ± 0.3	8.5 ± 1.0	9.2 ± 1.6
	30.6	1.1 ± 0.2	7.7 ± 2.0	9.0 ± 0.7
Oatp1a4 ^a	0	1.1 ± 0.1	53.1 ± 2.1	40.0 ± 5.5
	1.7	1.2 ± 0.0	54.0 ± 2.2	43.5 ± 4.4
	10.2	1.2 ± 0.1	57.4 ± 2.5	40.0 ± 3.8
	30.6	1.3 ± 0.1	69.4 ± 5.2**	42.9 ± 6.3
Ntcp ^a	0	1.2 ± 0.1	0.8 ± 0.1	1.0 ± 0.2
	1.7	1.2 ± 0.1	0.8 ± 0.1	1.0 ± 0.2
	10.2	1.2 ± 0.1	0.8 ± 0.1	1.0 ± 0.1
	30.6	1.1 ± 0.2	0.8 ± 0.1	1.0 ± 0.0

Note. Phase III hepatic influx transporters were measured for Oat2, Oatp1a4, and Ntcp by real-time PCR. RNA was isolated from male rat livers at PND 4, 21, and 60 after perinatal exposure to the polybrominated diphenyl ether mixture, DE-71 (0, 1.7, 10.2, and 30.6 mg/kg/day) from GD 6 to PND 21. Significantly different from control: ($p < 0.01$)* or ($p < 0.05$)**.

^aData are expressed as relative quantitation mean ± SD, $n = 5$.

interaction of age and dose for Oatp1a4 mRNA expression exists ($F_{6,48} = 7.540$; $p < 0.0001$). A *post hoc* test identified a 1.3-fold increase for 30.6 mg/kg/day at PND 21. No effect at PND 4 and 60 were observed for Oatp1a4.

Transthyretin: Gene Expression

There was a significant effect of age ($F_{6,48} = 205.7$; $p < 0.0001$) and dose ($F_{6,48} = 3.02$; $p < 0.039$) observed for Ttr (Table 7). A *post hoc* test indicated that Ttr mRNA expression was significantly decreased 20% at the highest dose tested, 30.6 mg/kg/day, on PND 21. No effects were seen at PND 4 or 60.

Deiodinase 1: Enzyme Activity and Gene Expression

D1 is a marker for acute changes in TH metabolism (Zoeller *et al.*, 2006) (Table 8). A significant interaction of age and dose

TABLE 7

Effect of DE-71 on Hepatic Transthyretin Gene Expression

Target	Treatment (mg/kg/day)	PND 4	PND 21	PND 60
Ttr ^a	0	1.0 ± 0.1	1.0 ± 0.1	0.4 ± 0.0
	1.7	1.0 ± 0.0	1.0 ± 0.1	0.4 ± 0.0
	10.2	0.9 ± 0.2	0.9 ± 0.1	0.4 ± 0.1
	30.6	0.9 ± 0.2	0.8 ± 0.2**	0.5 ± 0.0

Note. The serum binding protein, transthyretin, was measured by real-time PCR. RNA was isolated from male rat livers at PND 4, 21, and 60 after perinatal exposure to the polybrominated diphenyl ether mixture, DE-71 (0, 1.7, 10.2, and 30.6 mg/kg/day) from GD 6 to PND 21. Significantly different from control: ($p < 0.01$)* or ($p < 0.05$)**.

^aData are expressed as relative quantitation mean ± SD, $n = 5$.

TABLE 8
Effect of DE-71 on Hepatic Deiodinase 1 Protein Activity and Gene Expression

Target	Treatment (mg/kg/day)	PND 4	PND 21	PND 60
D1 ^a	0	257 ± 21	402 ± 99	500 ± 47
	1.7	111 ± 11*	312 ± 30	472 ± 56
	10.2	102 ± 10*	347 ± 32	489 ± 37
	30.6	114 ± 23*	115 ± 19*	492 ± 29
d1 ^b	0	1.7 ± 0.4	9.0 ± 0.9	0.5 ± 0.1
	1.7	1.6 ± 0.2	8.7 ± 0.6	0.4 ± 0.1
	10.2	1.1 ± 0.1**	8.3 ± 1.6	0.5 ± 0.2
	30.6	0.9 ± 0.3*	5.9 ± 1.3*	0.5 ± 0.1

Note. Deiodinase 1 enzyme activity was measured using T₄ as a substrate along with gene expression by real-time PCR. RNA was isolated from male rat livers at PND 4, 21, and 60 after perinatal exposure to the polybrominated diphenyl ether mixture, DE-71 (0, 1.7, 10.2, and 30.6 mg/kg/day) from GD 6 to PND 21.

Significantly different from control: ($p < 0.01$)* or ($p < 0.05$)**.

^aData are expressed as pmol/mg protein/min ± SD, $n = 5$.

^bData are expressed as relative quantitation mean ± SD.

for D1 enzyme activity ($F_{6,48} = 15.02$; $p < 0.0001$) was observed for all doses tested at PND 4 and for 30.6 mg/kg/day at PND 21. At PND 4, D1 activity decreased 60% at all doses, whereas at PND 21, a significant 70% decrease in activity was observed only at the highest dose. There was also a significant interaction of age and dose for d1 mRNA expression ($F_{6,48} = 5.978$; $p < 0.0001$). A *post hoc* test indicated an effect at doses of 10.2 and 30.6 mg/kg/day for PND 4 and at 30.6 mg/kg/day for PND 21, but not at PND 60. At PND 4, expression levels were decreased in a dose related fashion to 40 and 50% at 10.2 and 30.6 mg/kg/day, respectively. However, on PND 21, the only decrease in d1 expression was at the high dose. No changes were observed at PND 60.

DISCUSSION

Previous hypotheses on perinatal TH disruption by DE-71 have focused on induction of hepatic UGT-T₄-mediated TH catabolism resulting in decreased circulating T₄ levels. It has also been hypothesized that competitive binding between PBDE metabolites and TH to serum transport proteins affects thyroid homeostasis. This study further investigates parameters involved in the alteration of TH levels with a focus on nuclear receptor-mediated activation during development, in the presence of a PBDE mixture. Specifically, this study aims to further identify genes activated by AhR, CAR, and PXR during perinatal exposure to DE-71. This information will further the risk assessment and provide information as to the mechanisms by which this commercial penta mixture exerts its effects. Concomitant with a decrease in T₄, we observed an increase in hepatic UGT-T₄ activity and UGT mRNA isozyme gene

expression, a decrease in D1 enzyme activity and mRNA expression, and increases in the transcription of Sult1b1 and transporters of TH or glucuronides in neonatal and juvenile male rats following maternal PBDE treatment.

After perinatal exposure to DE-71, a dose-dependent induction of CYP1A1 and CYP2B demonstrates a similar induction pattern as previously reported (Zhou *et al.*, 2002), whereas we observe for the first time a dose-dependent induction of CYP3A. This is in agreement with a 28-day oral exposure study using a purified DE-71 mixture in adult Wistar rats (van der Ven *et al.*, 2008). This is also the first report measuring the induction of CYP450 mRNA expression after perinatal exposure to DE-71. The induction of CYP1A1, CYP2B, and CYP3A along with their respective mRNA transcripts signifies DE-71 is an agonist for AhR, CAR, and PXR, respectively. The increased induction seen in CYP2B as compared with CYP3A supports the suggestion that PBDE congeners have a preference for CAR over PXR (Pacyniak *et al.*, 2007; Sanders *et al.*, 2005; Richardson *et al.*, 2008). Although individual PBDE congeners have been shown not be AhR agonists (Peters *et al.*, 2006), induction of CYP1A1 gene expression and protein further supports the presence of dioxin-like contaminants in the DE-71 mixture (Hanari *et al.*, 2006) as being responsible for the Ah receptor-based responses (Sanders *et al.*, 2005).

Our data correlate with previous UGT developmental enzyme activity studies in Long-Evans male rats which also demonstrated decreases in TH concentrations along with increases in hepatic UGT-T₄ activity with exposure to DE-71 (Zhou *et al.*, 2002). Different UGT isoforms have unique developmental patterns and their regulation is thought to be nuclear receptor specific. In this study, perinatal DE-71 exposure increased AhR mediated Ugt1a6 (Auyeung *et al.*, 2003; Nishimura *et al.*, 2005) and Ugt1a7 (Metz and Ritter, 1998) along with CAR mediated Ugt2b (Zhou *et al.*, 2005) mRNA expression. Members of the UGT1A family specifically glucuronidate T₄, whereas members of the UGT2b family glucuronidate T₃ (Vansell and Klaassen, 2002). The significant increase in hepatic Ugt1a6 and Ugt1a7 mRNA expression further supports contaminants in DE-71 as agonists for the AhR. In addition, PBDE induction of Ugt2b at the high dose parallels the UGT induction pattern and is likely CAR mediated.

In this study, there is a lack of DE-71 effect on SULT-T₄, Sult1a1, and Sult1c1, whereas an increase in Sult1b1 mRNA expression was identified. SULT1A1, 1B1, and 1C1 have enzymatic activity towards T₃ and T₄, whereas SULT1C1 exhibits higher expression in males. The lack of a sex difference in Sult1b1 expression suggests it has a similar role in both sexes. For this reason it has been hypothesized that SULT1B1 may be more important for TH homeostasis than other isoforms (Dunn *et al.*, 1999; Fujita *et al.*, 1997). Considering sulfation is a reversible pathway of TH metabolism which depends on the free hormone recovery by sulfatases (Darras *et al.*, 1999), specific induction of Sult1b1 may be

important in TH homeostasis during exposure to endocrine disrupting compounds such as PBDEs. *In vitro* rodent experiments have demonstrated hepatic SULTs having different conjugation affinities for iodothyronines with $T_4 < rT_3 < T_3 < T_2$ (Kaptein *et al.*, 1997). Although the K_m of rodent SULT- T_4 is high in relation to other iodothyronines, T_4 comprises the fractional majority of TH at any given time in circulation and can possibly contribute to a large fraction of T_4 for sulfation. The lack of effect observed on hepatic SULT- T_4 activity after exposure to DE-71 contrasts with the observed increases in Sult1b1 mRNA expression. This could be due to SULT- T_4 enzyme activity assay not evaluating the activity of specific SULT isoforms. Alternatively, increases in Sult mRNA expression may not result in marked increases of the respective enzymes.

The efflux transporters Mdr1, Mrp2, and Mrp3 are members of the ABC binding cassette superfamily (Borst and Elferink, 2002; Dean and Allikmets, 2001) and are regulated by AhR, CAR and PXR (Cherrington *et al.*, 2002; Geick *et al.*, 2001; Johnson *et al.*, 2002; Kast *et al.*, 2002; Maglich *et al.*, 2002; Teng *et al.*, 2003; Xiong *et al.*, 2002). Mrp2 resides at the canalicular membrane and secretes its substrates into bile (Müller *et al.*, 1996). Mrp3 is present at low levels at the basolateral membrane for export of substrates into sinusoidal blood. The expression of Mdr1 and Mrp2 are inducible by hormones and steroids and their activity may change during development (Courtois *et al.*, 1999; Demeule *et al.*, 1999). The induction of Mdr1 and Mrp2 both occurred at PND 4 and 21 with Mdr1 having a greater sensitivity at PND 4. Although Mrp2 and Mdr1 both have a high sensitivity to PBDEs later in development, Mdr1 efflux mechanisms appear to be involved in elimination and detoxification during early postnatal development to a greater extent than Mrp2. The developmental expression of the basolateral efflux transporter Mrp3 has been previously documented in mice (Maher *et al.*, 2005) and correlates with our findings in rats. Its age and dose-dependent sensitivity to PBDEs appear to be similar to the canalicular efflux transporter Mdr1; however Mrp3 is induced to a greater degree.

In rat liver, basolateral uptake systems include the sodium taurocholate cotransport protein (NTCP) and the OATPs. The basolateral Na^+ -dependent bile salt transporter, NTCP, is specific to hepatocytes and is distributed homogeneously throughout the liver (Stieger *et al.*, 1994). Bile salts are the major substrate for NTCP, however, other compounds such as estrogen conjugates, TH, and xenobiotics that are covalently bound to taurocholate can also be transported (Kouzuki *et al.*, 2000). DE-71 did not alter mRNA expression levels of Ntcp. The lack of effect seen with exposure to this commercial mixture implies Ntcp is not activated by AhR/CAR/PXR. In addition, full maturation of NTCP transport activity is delayed until 4 weeks of age due to incomplete glycosylation (Kühkamp *et al.*, 2005). This delay may contribute to the lack of Ntcp mRNA expression alterations during perinatal exposure seen here.

TABLE 9
Summary on the Effect of DE-71 on THs, Hepatic Protein Activity, and Gene Expression^a

Target	PND 4	PND 21	PND 60
T_4	↓↓	↓↓	—
T_3	—	—	—
EROD	↑↑↑	↑↑↑	—
PROD	↑↑↑	↑↑	—
BROD	↑↑	↑↑↑	—
UGT- T_4	↑	↑	—
SULT- T_4	—	—	—
D1- T_4	↓↓↓	↓	—
Cyp1a1	↑↑↑	↑↑↑	↑
Cyp2b1	↑↑↑	↑↑↑	—
Cyp2b2	↑↑↑	↑↑↑	—
Cyp3a1	↑	↑↑	—
Ugt1a1	—	—	—
Ugt1a6	↑↑	—	—
Ugt1a7	↑	↑	—
Ugt2b	↑	↑↑	—
Sult1a1	—	—	—
Sult1b1	—	↑↑	—
Sult1c1	—	—	—
Mdr1	↑↑↑	↑↑↑	—
Mrp2	↑	↑↑↑	↓
Mrp3	↑↑↑	↑↑↑	—
Oatp1a4	—	↑	—
Oat2	—	—	—
Ntcp	—	—	—
Ttr	—	↓	—
d1	↓↓	↓	—

Note. Overall hepatic effects after perinatal exposure to the polybrominated diphenyl ether mixture, DE-71 on THs, hepatic protein activity and gene expression. Data was measured in male rat at PND 4, 21, and 60 after perinatal exposure to 0, 1.7, 10.2, and 30.6 mg/kg/day between GD 6 to PND 21.

^aThree, two, and one arrow refers to significant effects at doses 1.7, 10.2, and 30.6 mg/kg/day, respectively.

Although NTCP represents the major hepatocellular uptake system for conjugated bile salts, OATPs mediate sodium-independent uptake of a large variety of substrates. Specifically, OATP1a4 actively transports bile acids, xenobiotics, and TH. OATP1a4 belongs to the ABC cassette superfamily and is regulated by CAR and PXR (Wagner *et al.*, 2005). Because OATP1a4 is a basolateral hepatic influx transporter, the increased expression levels seen here may indicate a demand for sequestration of PBDEs and T_4 into the liver for biotransformation and elimination.

Oat2 is found in higher concentrations in adult male rat liver than in kidney, however, the opposite is true for females (Buist *et al.*, 2002; Pavlova *et al.*, 2000). In this study, the expression levels of Oat2 in male Long-Evans rats were low at PND 4 and increased at PND 21, and then remained high through PND 60. This developmental pattern is consistent with previous findings for rat Oat2 in the liver (Simonson *et al.*, 1994). The level of transcript increases

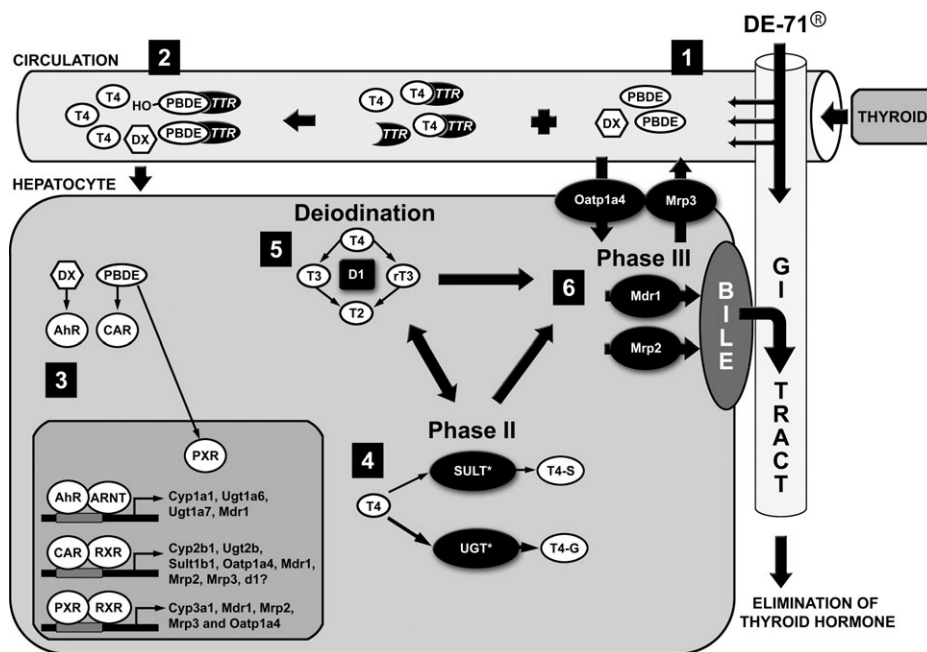


FIG. 1. Possible mechanisms of TH disruption after perinatal exposure to the commercial “penta” PBDE flame-retardant mixture, DE-71. (1) PBDE congeners and dioxin-like (DX) contaminants enter the circulation. (2) PBDE (parent or hydroxylated metabolite) displace T_4 from its serum binding protein, transthyretin (TTR). This result in an increase of free T_4 destined for hepatic metabolism and elimination. (3) PBDE and DX activate nuclear receptors initiating transcription of XMEs. (4) XMEs consequently conjugate T_4 by phase II enzymes, UGT and SULT. (5) Deiodinase 1 (D1) can deiodinate parent and conjugated T_4 along with their metabolites. (6) Influx transporters (Oatp1a4) further increase the uptake of T_4 for metabolism. Efflux transporters eliminate T_4 or its conjugates from hepatocytes either in the serum (Mrp3) or the bile (Mdr1 and Mrp2).

dramatically within two days post-partum and continues until the level stabilizes within the second week of postnatal development.

D1 is mainly expressed in the liver, thyroid, and kidney. Evidence suggests D1 is regulated directly or indirectly by CAR (Tien *et al.*, 2007) however time and dose response relationship observed here suggests D1 is regulated by CAR and/or AhR but not PXR. Raasmaja *et al.* (1996) suggest a possible mechanism for the reduction in T_4 to be due to increased tissue-specific deiodinase activity converting T_4 to T_3 . In this study, hepatic D1 activity decreased 60% at all doses on PND 4, and 70% with the highest dose on PND 21. Similar decreased serum T_4 and hepatic D1 enzyme activity were seen with the PCB mixture, Aroclor 1254 (Hood and Klaassen, 2000). Considering the similarities in PCB and PBDE structures and effects, common mechanisms are likely involved in D1 reduction. Interestingly, decreases in rat hepatic D1 have also been observed following exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and suggests D1 effects seen here may be attributed, at least in part, to the dioxin-like contaminants (Viluksela *et al.*, 2004). However, decreases in D1 were a secondary effect to circulating T_4 reduction after TCDD exposure. Future studies are needed to determine whether the sensitive decrease in D1 activity is also observed with exposure to purified PBDE congeners.

The reduction in D1 activity and mRNA message levels after perinatal exposure to the penta PBDE commercial mixture has

proven challenging to explain. D1 is thought to be responsible for the major portion of T_3 production peripherally. However, during hypothyroidism, plasma T_4 is reduced and peripheral T_3 conversion from T_4 is believed to be sustained by upregulation of extrahepatic D2 and downregulation of D1 (Zavacki *et al.*, 2005). D2 is a more catalytically efficient enzyme, maintaining T_3 levels by increasing the fractional conversion of T_4 to active T_3 as compared with the equal conversion of active T_3 and inactive rT_3 from D1.

In addition to D2, SULTs have been reported to work with D1 in the regulation of TH homeostasis. Sulfation in the human fetus has been proposed to be a protective mechanism regulating T_3 levels (Santini *et al.*, 1993). This regulation is mediated by deiodinases (T_4S to T_3S), and furthermore by sulfatases (T_3S to T_3), during periods of high T_3 demands (Chopra, 1994; Santini *et al.*, 1992). In this study, rat hepatic Sult1b1 was increased. It has been shown that deiodination rates by D1 of T_4 to inactive rT_3 are increased nearly 200 times by sulfation (T_4S to rT_3S), whereas deiodination of T_4 to active T_3 is completely lost after sulfation (T_4S to T_3S) (Visser *et al.*, 1993). Therefore, the decreases in rat hepatic D1 activity observed after exposure to xenobiotics may act as a protective mechanism to reduce the conversion of T_4S to inactive rT_3S . Favorably, this reduction of D1 serves to maintain T_3 levels.

Risk assessment of the PBDE commercial mixtures is ongoing and this *in vivo* study aids in a better evaluation of the

possible risks for human beings. Although the administered doses used here may seem high, this study as well as others, have shown that effects of PBDEs are seen in animal models at concentrations within 10-fold of the high end of the human population in North America (McDonald, 2005). This study demonstrates perinatal exposure as low as 1.7 mg DE-71/kg/day is sufficient to alter hepatic enzyme activity measured as early as PND 4 (Table 9). The most sensitive endpoints in terms of both mRNA and enzyme activity were a reduction of D1 and induction of CYP1A1, 2B1/2, and 3A. These endpoints were all more sensitive at PND 4 than PND 21 with the exception of Cyp3a1/CYP3A. In contrast, Ugt2b, Sult1b1, and Mrp2 expression are all higher on PND 21 than PND 4, but these endpoints were less sensitive than CYP3A. Expression of Mdr1 and Mrp3 are also extremely sensitive at PND 4. In contrast, UGT mRNA effects were only seen at 10.2 mg/kg/day or greater and enzyme activity at 30.6 mg/kg/day, indicating UGT-T₄ is not the most sensitive marker for this PBDE mixture. All effects were largely reversible by PND 60. The induction of hepatic Sult1b1 mRNA expression seen here along with decreases of D1 may work together to maintain serum T₃ and reduce T₄. In addition, the hepatic efflux transporters Mdr1, Mrp2, and Mrp3 may be involved. However, studies to identify sulfated TH specific transporters and whether the alterations in mRNA levels seen in this study are reflected in protein levels and enzyme activity are clearly needed.

The T₄ depleting effects of DE-71 are likely to involve multiple mechanisms of action including Phase II glucuronidation and sulfation, transthyretin displacement, decreased hepatic deiodinase 1 activity, and increases in hepatic transporter phase III elimination (Fig. 1). This study has demonstrated coordinate modification in the expression of transport proteins and detoxification enzymes in the postnatal period of development after low perinatal exposure to DE-71, a commercial PBDE mixture. These alterations could be responsible for the important and rapid changes in TH observed during this period of life. Because DE-71 contains both PBDEs and PBDDs/PBDFs, it is difficult to attribute every effect measured in this study to the sole activation of CAR/PXR or AhR, respectively. Considering household dust contains both PBDEs and dioxin-like compounds and is suspected to be a major source of exposure for humans and other animals, this commercial mixture study is important to the understanding of its real world toxic effects. In furthering the risk assessment of this commercial PBDE mixture, this data demonstrates that DE-71 disrupts TH homeostasis in rats during development via perinatal exposure; however, the mechanism(s) of action appear complex.

Supplementary Data

Supplementary data are available online at <http://toxsci.oxfordjournals.org/>.

FUNDING

National Institutes of Health (T32 ES007126); and Environmental Protection Agency (CR 833237) predoctoral training grants.

ACKNOWLEDGMENTS

This document has been reviewed by the National Health and Environmental Effects Research Laboratory. Approval does not denote that the contents of this document reflect the views of the Agency, nor does the mention of trade names or commercial products constitute endorsement or recommendation for use.

REFERENCES

- Auyeung, D. J., Kessler, F. K., and Ritter, J. K. (2003). Mechanism of rat UDP glucuronosyltransferase 1A6 induction by oltipraz: Evidence for a contribution of the Aryl hydrocarbon receptor pathway. *Mol. Pharmacol.* **63**, 119–127.
- Barter, R. A., and Klaassen, C. D. (1992). UDP-glucuronosyltransferase inducers reduce thyroid hormone levels in rats by an extrathyroidal mechanism. *Toxicol. Appl. Pharmacol.* **113**, 36–42.
- Birnbaum, L. S., Staskal, D. F., and Diliberto, J. J. (2004). Health effects of polybrominated dibenzo-p-dioxins (PBDDs) and dibenzofurans (PBDFs). *Environ. Int.* **6**, 855–860.
- Borst, P., and Elferink, R. O. (2002). Mammalian ABC transporters in health and disease. *Annu. Rev. Biochem.* **71**, 537–592.
- Buist, S. C., Cherrington, N. J., Choudhuri, S., Hartley, D. P., and Klaassen, C. D. (2002). Gender-specific and developmental influences on the expression of rat organic anion transporters. *J. Pharmacol. Exp. Ther.* **30**, 145–151.
- Cherrington, N. J., Hartley, D. P., Li, N., Johnson, D. R., and Klaassen, C. D. (2002). Organ distribution of multidrug resistance proteins 1, 2, and 3 (Mrp1, 2, and 3) mRNA and hepatic induction of Mrp3 by constitutive androstane receptor activators in rats. *J. Pharmacol. Exp. Ther.* **300**, 97–104.
- Chopra, I. J. (1994). The role of sulfation and desulfation in thyroid hormone metabolism. In *Thyroid Hormone Metabolism: Molecular Biology and Alternate Pathways* (S. Y. Wu and T. J. Visser, Eds.), pp. 119–138. CRC Press, Boca Raton, FL.
- Collins, W. T., Jr., and Capen, C. C. (1980). Biliary excretion of 125I-thyroxine and fine structural alterations in the thyroid glands of Gunn rats fed polychlorinated biphenyls (PCB). *Lab. Invest.* **43**, 58–64.
- Costa, L. G., and Giordano, G. (2007). Developmental neurotoxicity of polybrominated diphenyl ether (PBDE) flame retardants. *Neurotoxicology* **6**, 1047–1067.
- Courtois, A., Payen, L., Guillouzo, A., and Fardel, O. (1999). Up-regulation of multidrug resistance-associated protein 2 (MRP2) expression in rat hepatocytes by dexamethasone. *FEBS Lett.* **459**, 381–385.
- Damerud, P. O., Eriksen, G. S., Johannesson, T., Larsen, P. B., and Viluksela, M. (2001). Polybrominated diphenyl ethers: Occurrence, dietary exposure, and toxicology. *Environ. Health Perspect.* **109**, 49–68.
- Damerud, P. O., and Sinjari, T. (1996). Effects of polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PCBs) on thyroxine and TSH blood levels in rats and mice. *Organohalogen Compounds* **29**, 316–319.

- Darras, V. M., Hume, R., and Visser, T. J. (1999). Regulation of thyroid hormone metabolism during fetal development. *Mol. Cell. Endocrinol.* **151**, 37–47.
- Dean, M., and Allikmets, R. (2001). Complete characterization of the human ABC gene family. *J. Bioenerg. Biomembr.* **33**, 475–479.
- Demeule, M., Jodoin, J., Beaulieu, E., Brossard, M., and Béliveau, R. (1999). Dexamethasone modulation of multidrug transporters in normal tissues. *FEBS Lett.* **442**, 208–214.
- DeVito, M. J., Maier, W. E., Diliberto, J. J., and Birnbaum, L. S. (1993). Comparative ability of various PCBs, PCDFs, and TCDD to induce cytochrome P450 1A1 and 1A2 activity following 4 weeks of treatment. *Fundam. Appl. Toxicol.* **20**, 125–130.
- Dunn, R. T., 2nd, Gleason, B. A., Hartley, D. P., and Klaassen, C. D. (1999). Postnatal ontogeny and hormonal regulation of sulfotransferase SUL1B1 in male and female rats. *J. Pharmacol. Exp. Ther.* **290**, 319–324.
- Dunn, R. T., 2nd., and Klaassen, C. D. (2000). Thyroid hormone modulation of rat sulphotransferase mRNA expression. *Xenobiotica* **30**, 345–357.
- Ellis-Hutchings, R. G., Cherr, G. N., Hanna, L. A., and Keen, C. L. (2006). Polybrominated diphenyl ether (PBDE)-induced alterations in vitamin A and thyroid hormone concentrations in the rat during lactation and early postnatal development. *Toxicol. Appl. Pharmacol.* **215**, 135–145.
- Eriksson, P., Jakobsson, E., and Fredriksson, A. (2001). Brominated flame retardants: A novel class of developmental neurotoxicants in our environment? *Environ. Health Perspect.* **109**, 903–908.
- Friesema, E. C., Jansen, J., Milici, C., and Visser, T. J. (2005). Thyroid hormone transporters. *Vitam. Horm.* **70**, 137–167.
- Fujita, K., Nagata, K., Ozawa, S., Sasano, H., and Yamazoe, Y. (1997). Molecular cloning and characterization of rat ST1B1 and human ST1B2 cDNAs, encoding thyroid hormone sulfotransferases. *J. Biochem.* **122**, 1052–1061.
- Geick, A., Eichelbaum, M., and Burk, O. (2001). Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. *J. Biol. Chem.* **276**, 14581–14587.
- Goldey, E. S., Kehn, L. S., Lau, C., Rehnberg, G. L., and Crofton, K. M. (1995). Developmental exposure to polychlorinated biphenyls (Aroclor 1254) reduces circulating thyroid hormone concentrations and causes hearing deficits in rats. *Toxicol. Appl. Pharmacol.* **135**, 77–88.
- Hagenbuch, B. (2007). Cellular entry of thyroid hormones by organic anion transporting polypeptides. *Best Pract. Res. Clin. Endocrinol. Metab.* **21**, 209–221.
- Hallgren, S., and Darnerud, P. O. (2002). Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and chlorinated paraffins (CPs) in rats testing interactions and mechanisms for thyroid hormone effects. *Toxicology* **177**, 227–243.
- Hanari, N., Kannan, K., Miyake, Y., Okazawa, T., Kodavanti, P. R., Aldous, K. M., and Yamashita, N. (2006). Occurrence of polybrominated biphenyls, polybrominated dibenzo-p-dioxins, and polybrominated dibenzofurans as impurities in commercial polybrominated diphenyl ether mixtures. *Environ. Sci. Technol.* **40**, 4400–4405.
- Hood, A., and Klaassen, C. D. (2000). Effects of microsomal enzyme inducers on outer-ring deiodinase activity toward thyroid hormones in various rat tissues. *Toxicol. Appl. Pharmacol.* **163**, 240–248.
- Hooper, K., and McDonald, T. A. (2000). The PBDEs: An emerging environmental challenge and another reason for breast-milk monitoring programs. *Environ. Health Perspect.* **108**, 387–392.
- Johnson, D. R., Guo, G. L., and Klaassen, C. D. (2002). Expression of rat Multidrug Resistance Protein 2 (Mrp2) in male and female rats during normal and pregnenolone-16 α -carbonitrile (PCN)-induced postnatal ontogeny. *Toxicology* **178**, 209–219.
- Kaptein, E., van Haasteren, G. A., Linkels, E., de Greef, W. J., and Visser, T. J. (1997). Characterization of iodothyronine sulfotransferase activity in rat liver. *Endocrinology* **138**, 5136–5143.
- Kast, H. R., Goodwin, B., Tarr, P. T., Jones, S. A., Anisfeld, A. M., Stoltz, C. M., Tontonoz, P., Kliewer, S., Willson, T. M., and Edwards, P. A. (2002). Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. *J. Biol. Chem.* **277**, 2908–2915.
- Kato, Y., Ikushiro, S., Haraguchi, K., Yamazaki, T., Ito, Y., Suzuki, H., Kimura, R., Yamada, S., Inoue, T., and Degawa, M. (2004). A possible mechanism for decrease in serum thyroxine level by polychlorinated biphenyls in Wistar and Gunn rats. *Toxicol. Sci.* **81**, 309–315.
- Kester, M. H., Kaptein, E., Roest, T. J., van Dijk, C. H., Tibboel, D., Meinel, W., Glatt, H., Coughtrie, M. W., and Visser, T. J. (2003). Characterization of rat iodothyronine sulfotransferases. *Am. J. Physiol. Endocrinol. Metab.* **285**, 592–598.
- Kim, Y. J., Osako, M., and Sakai, S. (2006). Leaching characteristics of polybrominated diphenyl ethers (PBDEs) from flame-retardant plastics. *Chemosphere* **65**, 506–513.
- Kouzuki, H., Suzuki, H., Stieger, B., Meier, P. J., and Sugiyama, Y. (2000). Characterization of the transport properties of organic anion transporting polypeptide 1 (oatp1) and Na⁺/taurocholate cotransporting polypeptide (Ntcp): Comparative studies on the inhibitory effect of their possible substrates in hepatocytes and cDNA-transfected COS-7 cells. *J. Pharmacol. Exp. Ther.* **292**, 505–511.
- Kühlkamp, T., Keitel, V., Helmer, A., Häussinger, D., and Kubitz, R. (2005). Degradation of the sodium taurocholate cotransporting polypeptide (NTCP) by the ubiquitin-proteasome system. *Biol. Chem.* **386**, 1065–1074.
- Kuriyama, S. N., Wanner, A., Fidalgo-Neto, A. A., Talsness, C. E., Koerner, W., and Chahoud, I. (2007). Developmental exposure to low-dose PBDE-99: Tissue distribution and thyroid hormone levels. *Toxicology* **242**, 80–90.
- LaA Guardia, M. J., Hale, R. C., and Harvey, E. (2006). Detailed polybrominated diphenyl ether (PBDE) congener composition of the widely used penta-, octa-, and deca-PBDE technical flame-retardant mixtures. *Environ. Sci. Technol.* **40**, 6247–6254.
- Leonard, J. L., and Rosenberg, I. N. (1980). Iodothyronine 5'-deiodinase from rat kidney: Substrate specificity and the 5'-deiodination of reverse triiodothyronine. *Endocrinology* **107**, 1376–1383.
- Maglich, J. M., Stoltz, C. M., Goodwin, B., Hawkins-Brown, D., Moore, J. T., and Kliewer, S. A. (2002). Nuclear pregnane x receptor and constitutive androstane receptor regulate overlapping but distinct sets of genes involved in xenobiotic detoxification. *Mol. Pharmacol.* **62**, 638–646.
- Maier, J. M., Slitt, A. L., Cherrington, N. J., Cheng, X., and Klaassen, C. D. (2005). Tissue distribution and hepatic and renal ontogeny of the multidrug resistance-associated protein (Mrp) family in mice. *Drug Metab. Dispos.* **33**, 947–955.
- McDonald, T. A. (2005). Polybrominated diphenylether levels among United States residents: Daily intake and risk of harm to the developing brain and reproductive organs. *Integr. Environ. Assess. Manag.* **1**, 343–354.
- McDonald, T. A. (2002). A perspective on the potential health risks of PBDEs. *Chemosphere* **46**, 745–755.
- Meerts, I. A., van Zanden, J. J., Luijckx, E. A., van Leeuwen-Bol, I., Marsh, G., Jakobsson, E., Bergman, A., and Brouwer, A. (2000). Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro. *Toxicol. Sci.* **56**, 95–104.
- Metz, R. P., and Ritter, J. K. (1998). Transcriptional activation of the UDP-glucuronosyltransferase 1A7 gene in rat liver by aryl hydrocarbon receptor ligands and oltipraz. *J. Biol. Chem.* **273**, 5607–5614.
- Mitchell, A. M., Tom, M., and Mortimer, R. H. (2005). Thyroid hormone export from cells: Contribution of P-glycoprotein. *J. Endocrinol.* **185**, 93–98.

- Müller, M., Roelofsen, H., and Jansen, P. L. (1996). Secretion of organic anions by hepatocytes: Involvement of homologues of the multidrug resistance protein. *Semin. Liver Dis.* **16**, 211–220.
- Nishimura, N., Yonemoto, J., Miyabara, Y., Fujii-Kuriyama, Y., and Tohyama, C. (2005). Altered thyroxine and retinoid metabolic response to 2,3,7,8-tetrachlorodibenzo-p-dioxin in aryl hydrocarbon receptor-null mice. *Arch. Toxicol.* **79**, 260–267.
- Pacyniak, E. K., Cheng, X., Cunningham, M. L., Crofton, K., Klaassen, C. D., and Guo, G. L. (2007). The flame retardants, polybrominated diphenyl ethers, are pregnane X receptor activators. *Toxicol. Sci.* **97**, 94–102.
- Pavlova, A., Sakurai, H., Leclercq, B., Beier, D. R., Yu, A. S., and Nigam, S. K. (2000). Developmentally regulated expression of organic ion transporters NKT (OAT1), OCT1, NLT (OAT2), and Roct. *Am. J. Physiol. Renal Physiol.* **278**, F635–F643.
- Peters, A. K., Nijmeijer, S., Gradin, K., Backlund, M., Bergman, A., Poellinger, L., Denison, M. S., and Van den Berg, M. (2006). Interactions of polybrominated diphenyl ethers with the aryl hydrocarbon receptor pathway. *Toxicol. Sci.* **92**, 133–142.
- Raasmaja, A., Viluksela, M., and Rozman, K. K. (1996). Decreased liver type I 5'-deiodinase and increased brown adipose tissue type II 5'-deiodinase activity in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-treated Long-Evans rats. *Toxicology* **114**, 199–205.
- Ribeiro, R. C., Cavalieri, R. R., Lomri, N., Rahmaoui, C. M., Baxter, J. D., and Scharschmidt, B. F. (1996). Thyroid hormone export regulates cellular hormone content and response. *J. Biol. Chem.* **271**, 17147–17151.
- Rice, D. C., Reeve, E. A., Herlihy, A., Zoeller, R. T., Thompson, W. D., and Markowski, V. P. (2007). Developmental delays and locomotor activity in the C57BL6/J mouse following neonatal exposure to the fully-brominated PBDE, decabromodiphenyl ether. *Neurotoxicol. Teratol.* **29**, 511–520.
- Richardson, V. M., Staskal, D. F., Ross, D. G., Diliberto, J. J., Devito, M. J., and Birnbaum, L. S. (2008). Possible mechanisms of thyroid hormone disruption in mice by BDE 47, a major polybrominated diphenyl ether congener. *Toxicol. Appl. Pharmacol.* **226**, 244–250.
- Rooda, S. J., van Loon, M. A., and Visser, T. J. (1987). Metabolism of reverse triiodothyronine by isolated rat hepatocytes. *J. Clin. Invest.* **79**, 1740–1748.
- Sanders, J. M., Burka, L. T., Smith, C. S., Black, W., James, R., and Cunningham, M. L. (2005). Differential expression of CYP1A, 2B, and 3A genes in the F344 rat following exposure to a polybrominated diphenyl ether mixture or individual components. *Toxicol. Sci.* **88**, 127–133.
- Santini, F., Chopra, I. J., Wu, S. Y., Solomon, D. H., and Chua Teco, G. N. (1992). Metabolism of 3,5,3'-triiodothyronine sulfate by tissues of the fetal rat: A consideration of the role of desulfation of 3,5,3'-triiodothyronine sulfate as a source of T3. *Pediatr. Res.* **31**, 541–544.
- Santini, F., Cortelazzi, D., Baggiani, A. M., Marconi, A. M., Beck-Peccoz, P., and Chopra, I. J. (1993). A study of the serum 3,5,3'-triiodothyronine sulfate concentration in normal and hypothyroid fetuses at various gestational stages. *J. Clin. Endocrinol. Metab.* **76**, 1583–1587.
- Sher, E. S., Xu, X. M., Adams, P. M., Craft, C. M., and Stein, S. A. (1998). The effects of thyroid hormone level and action in developing brain: Are these targets for the actions of polychlorinated biphenyls and dioxins? *Toxicol. Ind. Health* **14**, 121–158.
- Simonson, G. D., Vincent, A. C., Roberg, K. J., Huang, Y., and Iwanij, V. (1994). Molecular cloning and characterization of a novel liver-specific transport protein. *J. Cell. Sci.* **107**, 1065–1072.
- Stapleton, H. M., Dodder, N. G., Offenberger, J. H., Schantz, M. M., and Wise, S. A. (2005). Polybrominated diphenyl ethers in house dust and clothes dryer lint. *Environ. Sci. Technol.* **39**, 925–931.
- Stieger, B., Hagenbuch, B., Landmann, L., Höchli, M., Schroeder, A., and Meier, P. J. (1994). In situ localization of the hepatocytic Na+/Taurocholate cotransporting polypeptide in rat liver. *Gastroenterology* **107**, 1781–1787.
- Teng, S., Jekerle, V., and Piquette-Miller, M. (2003). Induction of ABCC3 (MRP3) by pregnane X receptor activators. *Drug Metab. Dispos.* **31**, 1296–1299.
- Tien, E. S., Matsui, K., Moore, R., and Negishi, M. (2007). The nuclear receptor constitutively active/androstane receptor regulates type 1 deiodinase and thyroid hormone activity in the regenerating mouse liver. *Pharmacol. Exp. Ther.* **320**, 307–313.
- Tompkins, L. M., and Wallace, A. D. (2007). Mechanisms of cytochrome P450 induction. *J. Biochem. Mol. Toxicol.* **21**, 176–181.
- Tseng, L. H., Li, M. H., Tsai, S. S., Lee, C. W., Pan, M. H., Yao, W. J., and Hsu, P. C. (2008). Developmental exposure to decabromodiphenyl ether (PBDE 209): Effects on thyroid hormone and hepatic enzyme activity in male mouse offspring. *Chemosphere* **70**, 640–647.
- van der Ven, L. T., van de Kuil, T., Verhoef, A., Leonards, P. E., Slob, W., Cantón, R. F., Germer, S., Hamers, T., Visser, T. J., Litens, S., et al. (2008). A 28-day oral dose toxicity study enhanced to detect endocrine effects of a purified technical pentabromodiphenyl ether (pentaBDE) mixture in Wistar rats. *Toxicology* **245**, 109–122.
- Vansell, N. R., and Klaassen, C. D. (2002). Effect of microsomal enzyme inducers on the biliary excretion of triiodothyronine (T(3)) and its metabolites. *Toxicol. Sci.* **65**, 184–191.
- Viberg, H., Fredriksson, A., Jakobsson, E., Orn, U., and Eriksson, P. (2003). Neurobehavioral derangements in adult mice receiving decabrominated diphenyl ether (PBDE 209) during a defined period of neonatal brain development. *Toxicol. Sci.* **76**, 112–120.
- Viluksela, M., Raasmaja, A., Lebofsky, M., Stahl, B. U., and Rozman, K. K. (2004). Tissue-specific effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the activity of 5'-deiodinases I and II in rats. *Toxicol. Lett.* **147**, 133–142.
- Visser, T. J. (1994). Role of sulfation in thyroid hormone metabolism. *Chem. Biol. Interact.* **92**, 293–303.
- Visser, T. J., Kaptein, E., van Toor, H., van Raaij, J. A., van den Berg, K. J., Joe, C. T., van Engelen, J. G., and Brouwer, A. (1993). Glucuronidation of thyroid hormone in rat liver: Effects of in vivo treatment with microsomal enzyme inducers and in vitro assay conditions. *Endocrinology* **133**, 2177–2186.
- Wagner, M., Halilbasic, E., Marschall, H. U., Zollner, G., Fickert, P., Langner, C., Zatloukal, K., Denk, H., and Trauner, M. (2005). CAR and PXR agonists stimulate hepatic bile acid and bilirubin detoxification and elimination pathways in mice. *Hepatology* **42**, 420–430.
- Waxman, D. J. (1999). P450 gene induction by structurally diverse xenochemicals: Central role of nuclear receptors CAR, PXR, and PPAR. *Arch. Biochem. Biophys.* **369**, 11–23.
- Xie, W., Barwick, J. L., Downes, M., Blumberg, B., Simon, C. M., Nelson, M. C., Neuschwander-Tetri, B. A., Brunt, E. M., Guzelian, P. S., and Evans, R. M. (2000). Humanized xenobiotic response in mice expressing nuclear receptor SXR. *Nature* **406**, 435–439.
- Xiong, H., Yoshinari, K., Brouwer, K. L., and Negishi, M. (2002). Role of constitutive androstane receptor in the in vivo induction of Mrp3 and CYP2B1/2 by phenobarbital. *Drug Metab. Dispos.* **30**, 918–923.
- Xu, C., Li, C. Y., and Kong, A. N. (2005). Induction of phase, I, II and III drug metabolism/transport by xenobiotics. *Arch. Pharm. Res.* **28**, 249–268.
- Yamada, H., Ishii, Y., Yamamoto, M., and Oguri, K. (2006). Induction of the hepatic cytochrome P450 2B subfamily by xenobiotics: Research history, evolutionary aspect, relation to tumorigenesis, and mechanism. *Curr. Drug Metab.* **7**, 397–409.
- Zavacki, A. M., Ying, H., Christoffolete, M. A., Aerts, G., So, E., Harney, J. W., Cheng, S. Y., Larsen, P. R., and Bianco, A. C. (2005).

- Type 1 iodothyronine deiodinase is a sensitive marker of peripheral thyroid status in the mouse. *Endocrinology* **146**, 1568–1575.
- Zhou, J., Zhang, J., and Xie, W. (2005). Xenobiotic nuclear receptor-mediated regulation of UDP-glucuronosyl-transferases. *Curr. Drug Metab.* **6**, 289–298.
- Zhou, T., Ross, D. J., DeVito, M. J., and Crofton, K. M. (2001). Effects of short-term *in vivo* exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic enzyme activities in weanling rats. *Toxicol. Sci.* **61**, 76–82.
- Zhou, T., Taylor, M. M., DeVito, M. J., and Crofton, K. M. (2002). Developmental exposure to brominated diphenyl ethers results in thyroid hormone disruption. *Toxicol. Sci.* **66**, 105–116.
- Zoeller, R. T., Tan, S. W., and Tyl, R. W. (2006). General background on the hypothalamic-pituitary-thyroid (HPT) axis. *Crit. Rev. Toxicol.* **37**, 11–53.